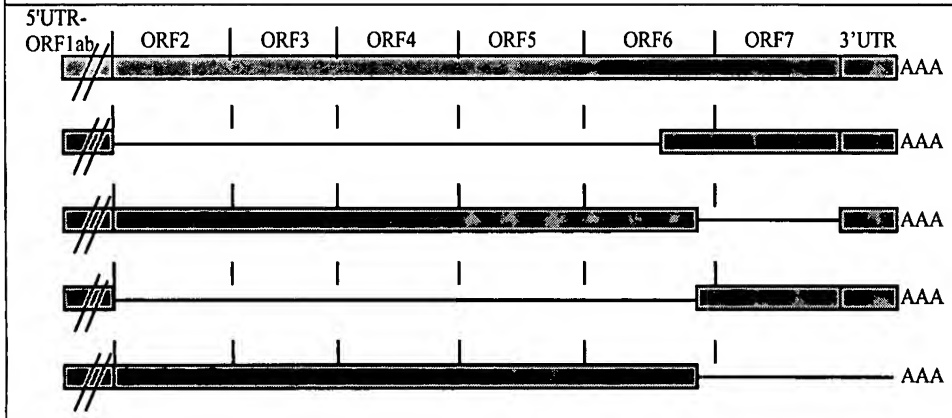
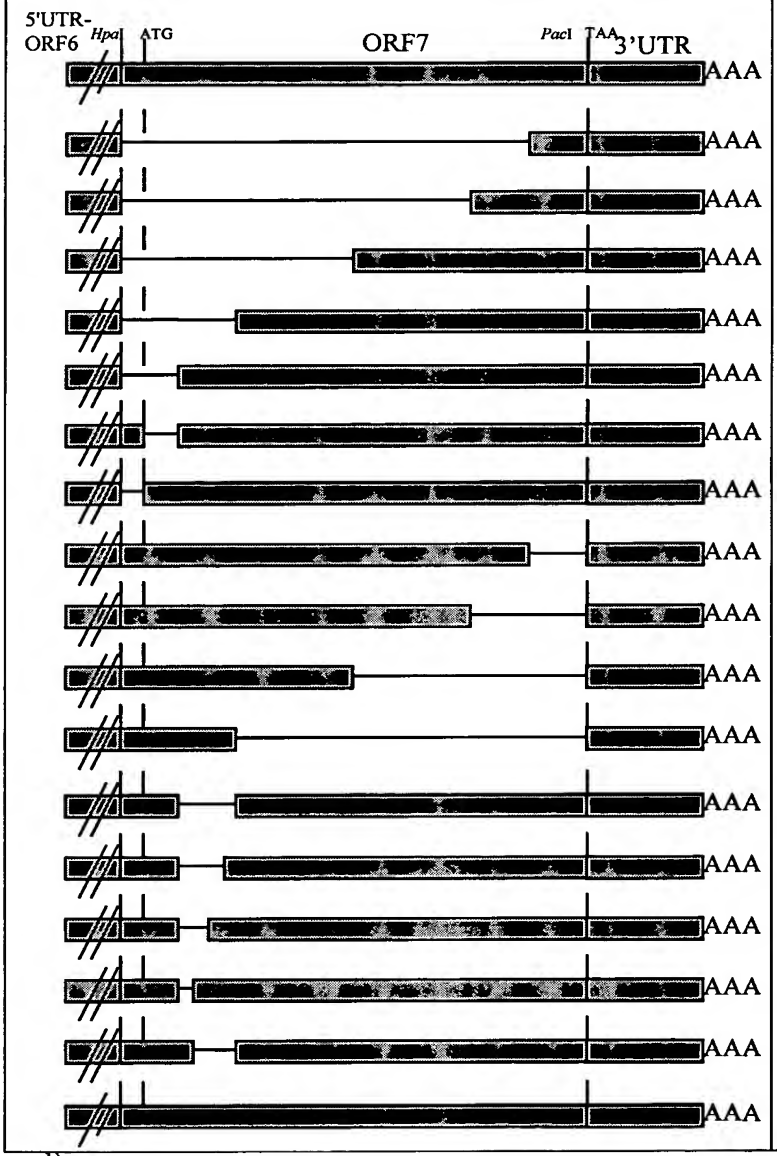
Constructs	Deletion (nucleotides)	pABV number	M-expression	N-expression
	-	437	+	+
	Δ 11788-14139	594	-	+
	Δ 14585-14984	521	-[1)]	-
	Δ 11788-14584	664	-	+
	Δ 14985-15111	668	-[1)]	-

[1)] Identical results were obtained in IPMA using MAbs against GP_3 and GP_4

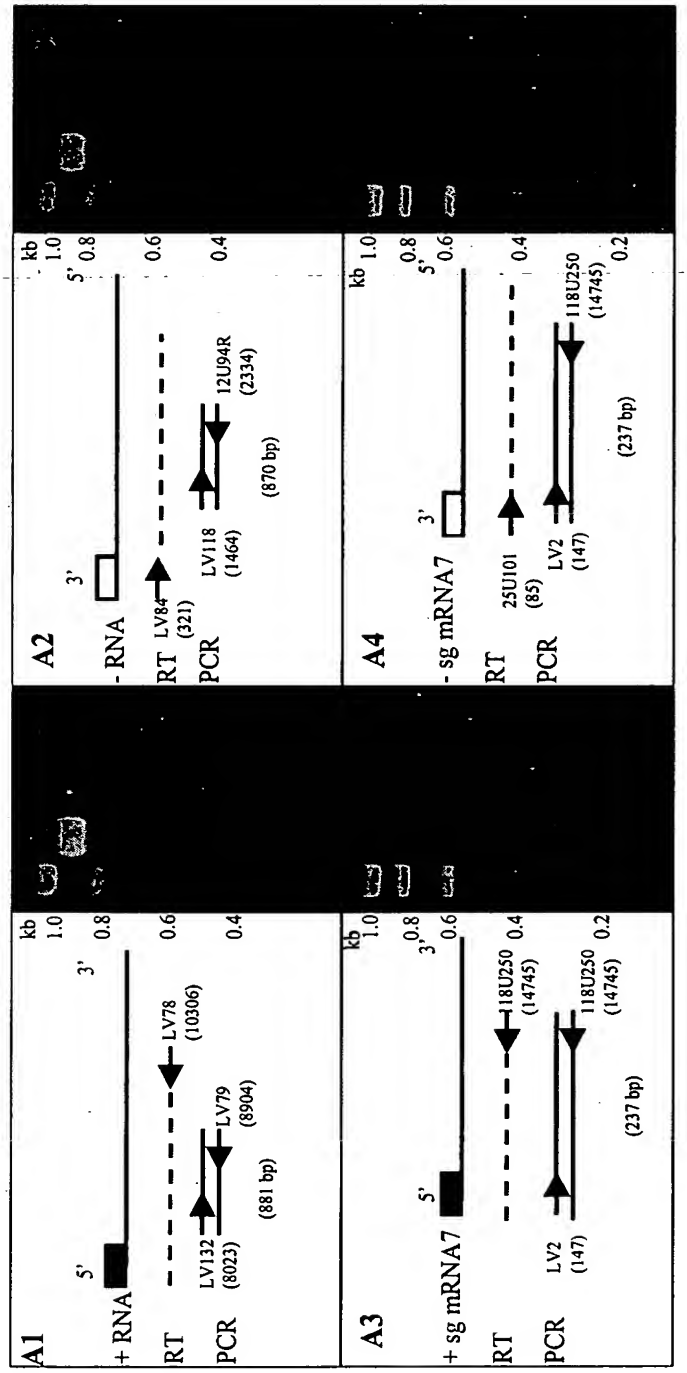
Fig. 1A

Constructs	Deletion (nucleotides)	PABV number	M-expression
	-	437	+[1)]
	Δ 14588-14936	605	-
	Δ 14588-14885	604	-
	Δ 14588-14786	603	-
	Δ 14588-14687	602	-
	Δ 14588-14642	624	+
	Δ 14599-14642	625	+
	Δ 14588-14600	626	+[1)]
	Δ 14938-14980	638	+[1)]
	Δ 14887-14980	637	+
	Δ 14788-14980	636	+
	Δ 14686-14980	635	+
	Δ 14643-14686	631	-
	Δ 14643-14676	632	-
	Δ 14643-14664	633	-
	Δ 14643-14652	634	+
	Δ 14653-14686	696	-
	rescue of 696	730	+[1)]

[1)] Identical results were obtained in IPMA using MAb 122.17 against N

Fig. 1B

Fig. 2


A1
+ RNA
5'
3'
RT
LV132 (8023)
LV79 (8904)
LV78 (10306)
PCR
(881 bp)
A2
- RNA
3'
5'
RT
LV84 (321)
PCR
LV118 (1464)
12U94R (2334)
(870 bp)
A3
+ sg mRNA7
5'
3'
RT
118U250 (14745)
PCR
LV2 (147)
118U250 (14745)
(237 bp)
A4
- sg mRNA7
3'
5'
RT
25U101 (85)
PCR
LV2 (147)
118U250 (14745)
(237 bp)
kb
1.0
0.8
0.6
0.4
0.2

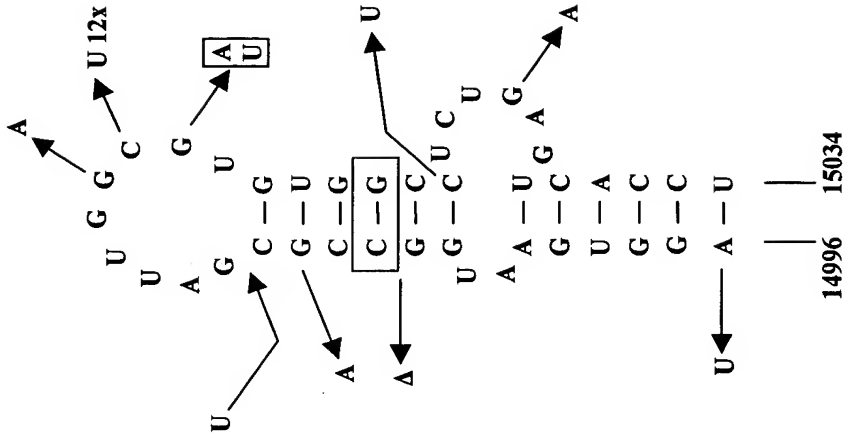


Fig. 3B

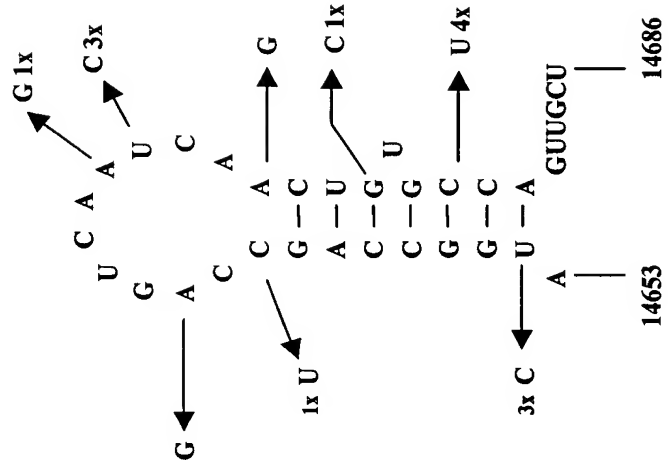
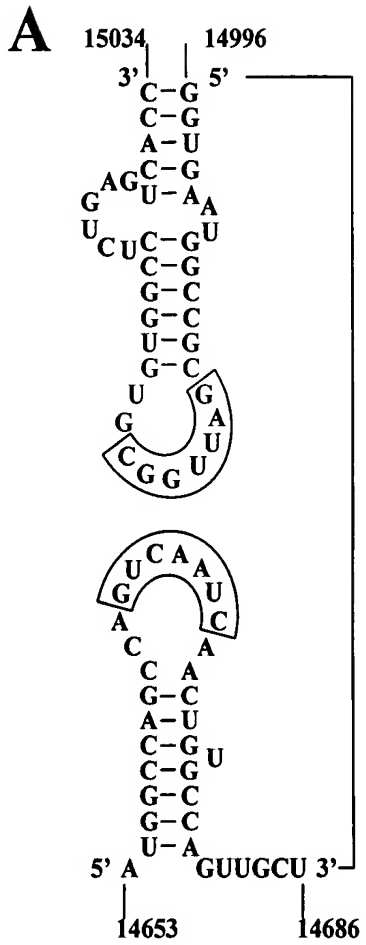


Fig. 3A


A
15034
14996
3'
5'
5' A
GUUGCU 3'
14653
14686

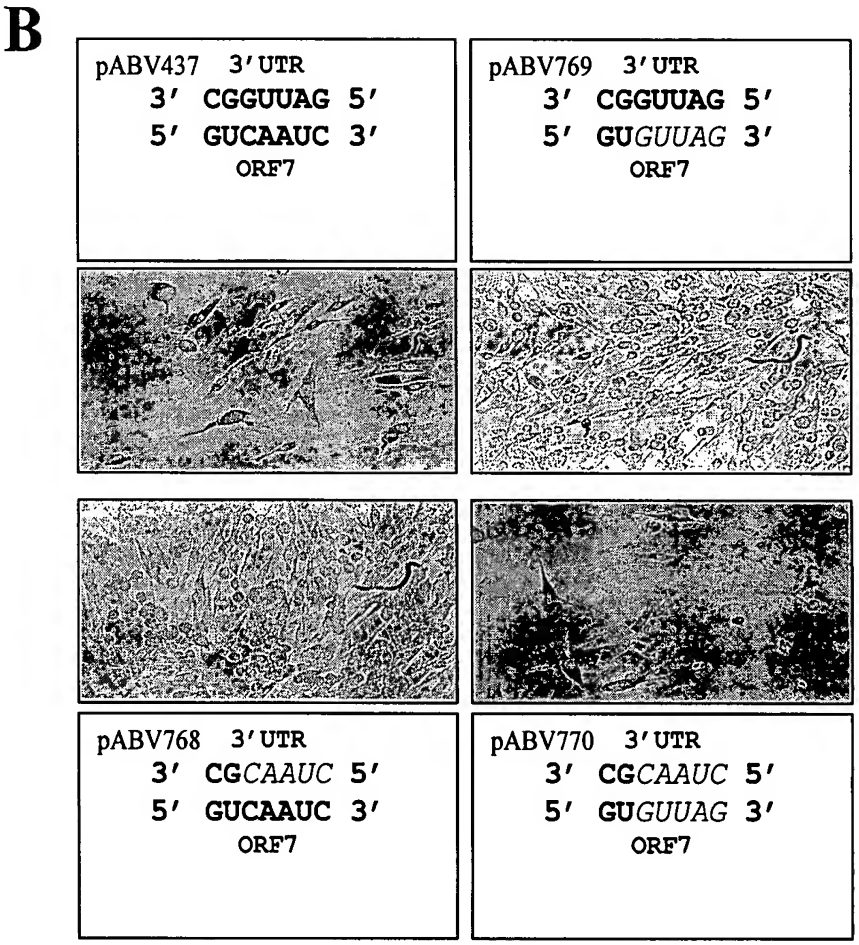

B
pABV437 3'UTR
3' CGGUUAG 5'
5' GUCAAUC 3'
ORF7
pABV769 3'UTR
3' CGGUUAG 5'
5' GUGUUAG 3'
ORF7
pABV768 3'UTR
3' CGCAAUC 5'
5' GUCAAUC 3'
ORF7
pABV770 3'UTR
3' CGCAAUC 5'
5' GUGUUAG 3'
ORF7

Fig. 4

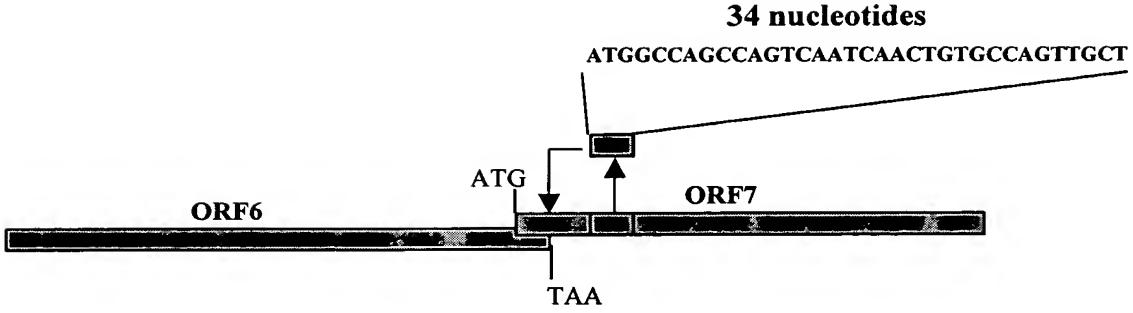

34 nucleotides
ATGGCCAGCCAGTCAATCAACTGTGCCAGTTGCT
ATG
ORF6
ORF7
TAA

Fig. 5

A

```
LV     : MAGKNQSQKKKKSTAPMGNGQPVNQLCQLLGAMIKSQRQ---QPRGGQAKKKKPEKPHFPLAAEDDIRHH :  67
VR2332 : MPNNNGKQQKRK----KGDGQPVNQLCQMLGKIIAQQNQSRGKGPGKKNKKKNPEKPHFPLATEDDVRHH :  66
           *   *  * * *     * ********* **  *  * *      *   *** ********* *** ***


LV     : LTQTERSLCLQSIQTAFNQGAGTASLSSSGKVSFQVEFMLPVAHTVRLIRVTSTSASQGAS : 128
VR2332 : FTPSERQLCLSSIQTAFNQGAGTCTLSDSGRISYTVEFSLPTHHTVRLIRVTASPSA---- : 123
           *  ** *** ************  ** **  *  *** **  *********
```

B

```
LV     : TTAAACAGTCA-------------------------------------GGTGAATGGCCGCGATTGGCG :  32
VR2332 : TGGGCTGGCATTCTTGAGGCATCTCAGTGTTTGAATTGGAAGAATGTGTGGTGAATGGCACTGATTGACA :  70
           *  *   *                                        **********   ***** *


LV     : TGTGGCCTCTGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTCATACTTAATCAGGCAGGAACCAT : 102
VR2332 : TTGTGCCTCTAAGTCACCTATTCAATTAGGGCGACCGTGTGGGGGTGAGATTTAATT-GGCGAGAACCAT : 139
           *   ****** ********************** *   ******* * * *****  ***  ******


LV     : GTGACCGAAATTAAAAAAAA: 122
VR2332 : GCGGCCGAAATTAAAAAAAA: 159
           * * ***************
```

Fig. 6

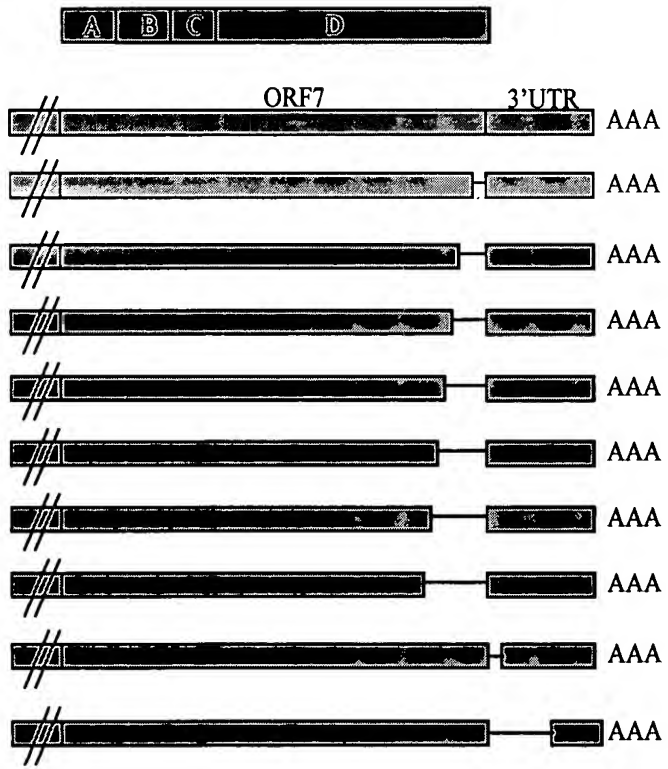
Constructs	Deletion (nucleotides / amino acids)	Plasmid number	M-expression	N-expression	Virus production
	wild type	437	+	+	+
	Δ14975-14980 / Δ 2	639	+	+	+
	Δ14969-14980 / Δ 4	694	+	+	+
	Δ14966-14980/ Δ 5	745	+	+	+
	Δ14963-14980/ Δ 6	746	+	+	+
	Δ14960-14980/ Δ 7	747	+	+	-
	Δ14957-14980/ Δ 8	748	+	+	-
	Δ14954-14980/ Δ 9	695	+	+	-
	Δ14989-14995	693	+	+	+
	Δ14989-15020	729	-	-	-

Fig. 7

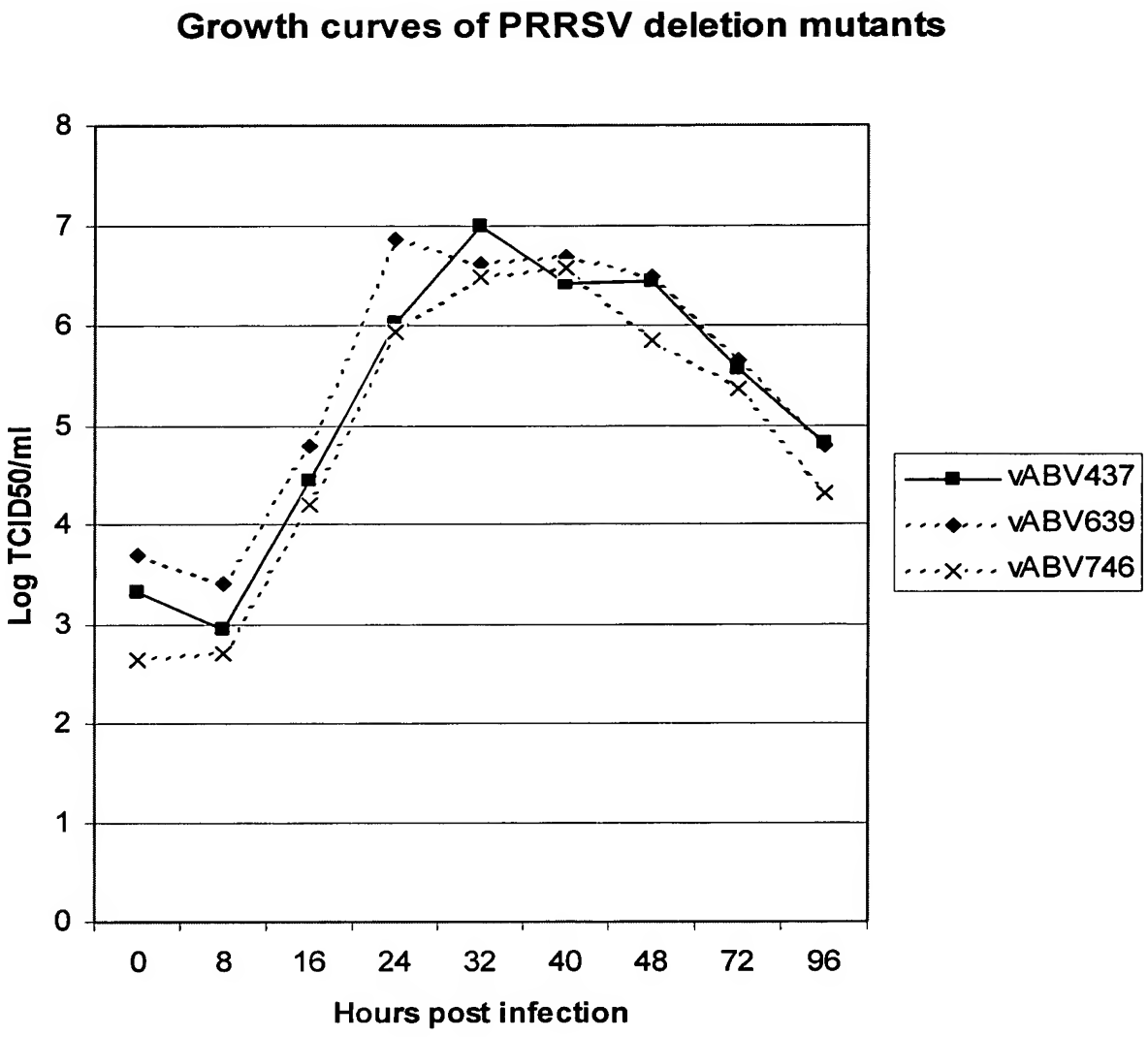

Growth curves of PRRSV deletion mutants
Log TCID50/ml
8
7
6
5
4
3
2
1
0
0
8
16
24
32
40
48
72
96
Hours post infection
vABV437
vABV639
vABV746

Fig. 8

Fig. 9

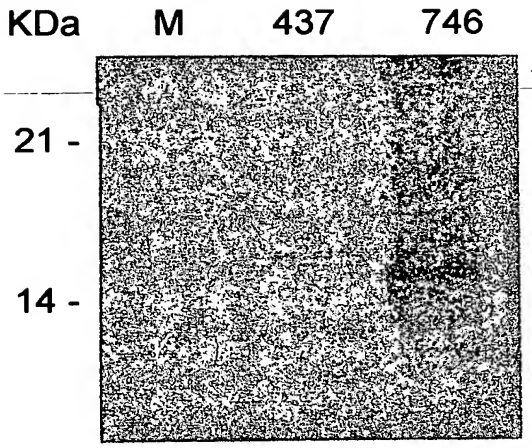


Fig. 10

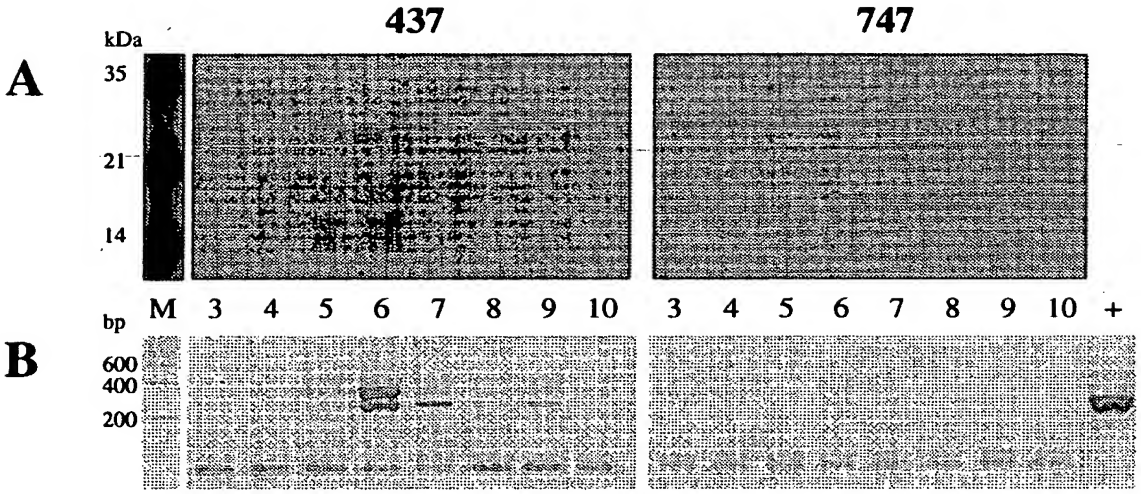


TABLE 1: Sequences of the primers used to introduce deletions by PCR, and primers used to sequence the introduced mutations.

Primer	Sequence of the primer[a]	Orientation	Purpose (pABV)	Location
119R218R	5' ATGACATCCGGCACCACC 3'	+	Sequencing	14782
LV20	5' CCTGATTAAAAGCTTGACCCC 3'	-	Sequencing	15066
LV75	5' TCTAGGAATTCTAGACGATCG 3'	-	*Xba*I –site	15088
LV155	5' ACGTGCGTTAACCTCGTCAAGTATGGCCGGTAAAAACCAGAGCCAGA 3'	+	*Hpa*I-site	14582
LV204	5' ACGTGCTTAATTAACCTTGACTGGCGGATGTAGA 3'	-	639	14974
LV213	5' TGCAAGTTAATTAAGGTGAATGGCCGCGA 3'	+	693	14996
LV 214	5' GACTGTTTAATTAACTGGCGGATGTA 3'	-	694	14958
LV215	5' GACTGTTTAATTAAGTCACGCGAATC 3'	-	695	14942
LV239	5' TGCAAGTTAATTAAGCCTCTGAGTCA 3'	+	729	15021
LV263	5' GACTGTTTAATTAAGCGGATGTAGA 3'	-	745	14954
LV264	5' GACTGTTTAATTAAGATGTAGAAGTC 3'	-	746	14951
LV265	5' GACTGTTTAATTAAGTAGAAGTCACG 3'	-	747	14948
LV266	5' GACTGTTTAATTAAGAAGTCACGCGA 3'	-	748	14945

[a] The restriction sites are underlined.

Fig. 11

TABLE 1: Sequences of the primers used to introduce deletions by PCR, primers used to sequence the introduced mutations, and primers used for the strand-specific RT-PCR

Primer	Sequence of the primer[a]	Orien-tation	Purpose (pABV)	Location
118U250	5' CAGCCAGGGGAAAATGTGGC 3'	-	Sequencing / Strand-sp. PCR	14745
12U94R	5' CACCTGTACCTGCTCATTGT 3'	-	Strand-sp. PCR	2334
25U101	5' GTTCTAGCCCAACAGGTATC 3'	+	Strand-sp. RT	85
LV2	5' AGCGGGAAGGATCCACCGAGTAT 3'	+	Strand-sp. PCR	147
LV17	5' CCCTTGACGAGCTCTTCGGC 3'	+	Sequencing	14045
LV20	5' CCTGATTAAAAGCTTGACCCC 3'	+	Sequencing	15066
LV75	5' TCTAGGAATTCTAGACGATCG	-	PCR *Xba*I –site	15088
LV76	5' TCTAGGAATTCTAGACGATCG(T)40 3'	-	RT	15088
LV78	5' CCCTGGGATGAATCTATGGT 3'	-	Strand-sp. RT	10306
LV79	5' GACAAGATCATCAGAGTATACC 3'	-	Strand-sp. PCR	8904
LV84	5' AGAGCTTCAGGACACTGACC 3'	+	Strand-sp. RT	321
LV112	5' CCATTCACCTGACTGTTTAATTAACTTGCACCCTGA 3'	-	PCR *Pac*I -site	14981
LV118	5' TTACCACCTACTCTCCACCG 3'	+	Strand-sp. PCR	1464
LV132	5' CCTACTGTGCCCTATAGTGTC 3'	+	Strand-sp. PCR	8023
LV151	5' ACCAGAGCCAGAAGAAAAAGAAAAGTACAGCTGGGTGCAATGAT 3'	+	PCR (631)	14611
LV152	5' ACCAGAGCCAGAAGAAAAAGAAAAGTACAGCTGCCAGTTGCTGG 3'	+	PCR (632)	14611
LV153	5' ACCAGAGCCAGAAGAAAAAGAAAAGTACAGCTTCAATCAACTGT 3'	+	PCR (633)	14611
LV154	5' ACCAGAGCCAGAAGAAAAAGAAAAGTACAGCTATGGCCAGCCAG 3'	+	PCR (634)	14611
LV155	5' ACGTGCGTTAACCTCGTCAAGTATGGCCGGTAAAAACCAGAGCCAGA 3'	+	*Hpa*I-site PCR	14582
LV188	5' ACGTGCGTTAACTAAGGTGCAATGATAAAGTCCCA 3'	+	PCR (602)	14582
LV189	5' ACGTGCGTTAACTAAATCCCGGCACCACCTCACCCA 3'	+	PCR (603)	14582
LV190	5' ACGTGCGTTAACTAAGGGAAGGTCAGTTTTCAGGT 3'	+	PCR (604)	14582
LV191	5' ACGTGCGTTAACTAACGCCTGATTCGCGTGACTTC 3'	+	PCR (605)	14582
LV195	5' ACGTGCGTTAACTAACCGATGGGGAATGGCCAG 3'	+	PCR (624)	14582
LV196	5' GGAGTGGTTAACCTCGTCAAGTAACCGATGGGGAATGGCCAG 3'	+	PCR (625)	14582
LV197	5' ACGTGCGTTAACGGCCGGTAAAAACCAGAGC 3'	+	PCR (626)	14582
LV198	5' GCTCGTGCTAGCCTTTAGCATCACATACAC 3'	+	*Nhe*I –site PCR	14140
LV200	5' ACGTGCTTAATTAACCCAGCAACTGGCACAGTTG 3'	-	PCR (635)	14981
LV201	5' ACGTGCTTAATTAAATGTCATCTTCAGCAGCCAG 3'	-	PCR (636)	14981
LV202	5' ACGTGCTTAATTAACCGCTGGATGAAAGCGACGC 3'	-	PCR)637)	14981
LV203	5' ACGTGCTTAATTAACGCACTGTATGAGCAACCGG 3'	-	PCR (638)	14981
LV204	5' ACGTGCTTAATTAACCTTGACTGGCGGATGTAGA 3'	-	PCR (639)	14981
LV216	5' ACCAGAGCCAGAAGAAAAAGAAAAGTACAGCTCCGATGGGGAG GGTGCAATGAT 3'	+	PCR (696)	14611
LV268	5' ACCAGAGCCAGAAGAAAAAGAAAAGTACAGCTCCGATGGGGA 3'	+	PCR (769)	14611
LV269	5' CTCCGATGGGGAATGGCCAGCCAGTGTTAGAACTGTGCCAGT 3'	+	PCR (769)	14641
LV270	5' TGCAAGTTAATTAAACAGTCAGGTGAATGGCCGCCTAACGCGTGTGGCCTC 3'	+	PCR (768)	14981

[a]The restriction sites are underlined.

Fig. 12